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Overview of Special Session B—Compositional and Structural Analysis of Biomass

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Abstract Special Session B at the 29th Symposium on Biotechnology for Fuels and Chemicals was the first invited session at this symposium devoted to analytical methods. The special topic was added in response to numerous requests for information on new and innovative methods that could be applied in the growing renewable fuels industry. Presentation topics include analytical methods for the characterization and analysis of maize traits, tools for investigating cell wall limitations to enzymatic degradation, methods for customizing enzyme cocktails for biomass, new techniques for the analysis of carbohydrates, analytical methods that enhance our understanding of pretreatment, improved methods for monitoring process intermediates, and published standard analytical methods for biomass conversion processes.

Keywords Analytical chemistry · Enzymes · Chromatography · Standard methods · Feedstock · Biomass · Forage · Carbohydrates · Imaging · Pretreatment · Saccharification · Process monitoring

Special Session B at the 29th Symposium on Biotechnology for Fuels and Chemicals Invited was the first session at this symposium devoted entirely to analytical methods. The special topic was added in response to numerous requests for information on new and innovative methods that could be applied in the growing renewable fuels industry. Quality analytical methods are needed in all areas of biomass conversion. Feedstock methods support breeding and agronomic programs that improve yields and quality. Process methods improve our understanding of pretreatment, saccharification, fermentation, combustion, and recycle steps. Imaging tools provide new insight into recalcitrance and inhibition. Methods that retain precision and accuracy but enhance sample throughput are being developed in many forms. This session captured a diverse cross section of the innovative analytical methods supporting biotechnology approaches to fuels and chemicals production. The

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following excerpts from presentation abstracts help summarize the diverse topics presented in this inaugural session on analytical methods

The session opened with a presentation by Dr. Brigette Chabbert, research scientist at *Institut National de la Recherche Agronomique*, *Unite Mixte de Recherche*, in Reims, France, who discussed "Cell Wall Limitations to Efficient Lignocellulosic Bioconversion."

> Improvement and development of efficient enzymatic tools for biomass conversion require in depth knowledge of the main limitations brought by lignified cell-walls. Non-invasive methods such (immuno)cytochemistry provide specific information on the cell wall heterogeneity at both cellular and subcellular levels. Notably, complexity of the cell wall networks can be clearly evidenced following enzymatic destructuration. Enzyme engineering aimed at modulating enzyme size and activity can provide a variety of molecular tools that will allow significant knowledge of the organizational heterogeneity of plant cell walls. Strategy based on in muro probing of the wall network in plant tissues would take advantage from comparative studies on various botanical plant materials. The use of in vitro systems of the lignin-polysaccharide matrix provides information on the way supramolecular organization of the wall polymers may affect the enzymatic breakdown of lignocellulosics.

Dr. Justin Stege, Associate Director of Alternative Fuels at Diversa in San Diego, CA, presented "Analytical Techniques for Enzyme Cocktails."

> Effective saccharification of pretreated biomass requires the synergistic activities of multiple enzymes, particularly when significant hemicellulose structures remain. With over 1000 plant cell wall-degrading enzymes in our collection, Diversa develops cocktails of enzymes that are customized for each particular feedstock, pretreatment and for different process conditions. Diversa has developed a 96-well capillary electrophoresis system for profiling oligosaccharide and monosaccharide reaction products. This high throughput CE technology enables us to rapidly screen hundreds of enzymes or enzyme combinations under different conditions with different substrates to select for desirable product profiles. These customized cocktails can be further improved by analyzing the undigested carbohydrates remaining after enzyme digestion. Using this compositional information, enzymes have been identified that saccharify the resistant material, greatly improving the yield of fermentable sugars.

Dr. Neil Price, from the USDA National Center for Agricultural Utilization Research in Peoria, IL, presented, "New Techniques for the Analysis of Carbohydrates."

> Carbohydrates are complex. In addition to the size/mass differences increasing from monosaccharides to oligosaccharides to polysaccharides, the carbohydrate analyst must also contend with which sugars are present (composition), how they are joined together (linkage), and their stereochemistry (configuration and anomericity). New techniques developed for glycomics and glycoproteomics are highly applicable for the analysis of most complex carbohydrate products. This talk reviewed methods for composition and linkage analysis of sugars, and introduced new methods of end-labeling, chiral isotopic labeling, and deuterium exchange (HX) that are compatible with MALDI-TOF MS, electrospray mass spectrometry, and NMR for the analysis of carbohydrates.

Dr. David Johnson, from the National Renewable Energy Lab, in Golden, CO, gave an overview of "Methods that Enhance our Understanding of Pretreatment Processes."

Conversion of lignocellulosic biomass to sugars and ethanol requires an effective pretreatment before the cellulose can be efficiently hydrolyzed by enzymes. Pretreatment conditions cover the entire range from low to high pH, from moderate to high temperatures, and from minutes to weeks. As with all chemical processes, pretreatments are only successful if they generate the desired products in high yield and undesirable products are minimized. Development of tools to understand the effect of pretreatment processes on lignocellulosic feedstocks is an active area of research at NREL. Characteristics typically tracked are cellulose crystallinity (by solid-state 13C NMR or X-ray diffraction), cellulose accessibility (measured using fluorescence labeled enzymes) and porosity (measured by thermoporometry or solute exclusion). Various microscopic imaging techniques can be used to follow changes in lignin and xylan distribution in the plant cell wall. By labeling with carbohydrate specific probes changes in the cell wall structure can be revealed. Immunoelectron microscopy has been used to monitor how major enzyme components of biomass degrading enzyme cocktails penetrate the cell wall matrix following pretreatment. Using these tools, we are attempting to gain a better understanding of how pretreatment processes can generate highly digestible.

The session's student speaker, Aaron Lorenz, a doctoral candidate in the Department of Agronomy at the University of Wisconsin in Madison, discussed "Silage Breeding Programs and their Connection to Energy Feedstock Production."

The University of Wisconsin has conducted a silage breeding program for the past 15 years using NIRS-based predictions of forage quality. A review of the development and operation of this breeding program provides some perspective on the future of lignocellulosic biofeedstock development programs. Evaluation of biomass yield and composition, followed by recombination of selected genotypes require logistical efficiency if significant gains are to be realized in the near future. Prior experience with measures of forage quality (e.g., fiber concentration and forage digestibility) may help if these relate well to requirements for lignocellulosic ethanol production, particularly with respect to the characteristics of the germplasm used to initiate genetic improvement programs.

Dr. Kevin Chamblis, Assistant Professor in the Department of Chemistry and Biochemistry, at Baylor University in Waco, TX, described "Improved Analytical Methods for Monitoring Process Intermediates in Biomass-to-Ethanol Conversion."

Qualitative and quantitative analysis of lignocellulosic degradation products is critical to any technical or economic valuation of biomass conversion. The majority of degradation products are introduced into process streams during biomass pretreatment. Pretreatment liquids contain not only cellulose and fermentable sugars, but also a wide variety of aliphatic and aromatic acids, phenols, and aromatic aldehydes that may exert an inhibitory effect on downstream bioconversion processes. Accordingly, there is broad interest in the development of reliable methods for assessment of an increasing number of target analytes in biomass process streams.

This presentation highlighted recent advances in the development of a high performance liquid chromatography-photodiode array-tandem mass spectrometry (HPLC-PDA-MS/MS) method, targeting 40 potentially-inhibitory degradation products, and an improved high performance anion exchange-pulsed amperometry (HPAE-PAD) technique for rapid monitoring of biomass sugars in pretreatment liquids.

Closing the session, Dr Foster Agblevor, Associate Professor in the Department of Biological Systems Engineering at Virginia Tech in Blacksburg, described "ASTM E48 Activities in the Area of Standard Analytical Methods Supporting Biomass Conversion Processes."

> Realizing the goals of the US DOE 30x'30 initiative will require the development, standardization and validation of hundreds of new analytical methods specifically for biomass. The data generated from these analytical methods will be used to evaluate feedstocks, optimize reactors and determine process economics for bench-scale to commercial-scale processes that convert biomass feedstocks to liquid transportation fuels. Efficient utilization of the slate of biomass feedstocks proposed in the DOE Billion Ton Study will require a multitude of individual analytical methods capable of tracking as many as 20 constituents from feedstock to products.
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> Deployment of biomass analysis into the private sector will more likely be in the form of high-throughput methods that will be calibrated using, and validated relative to, the best chemical and structural tools available, providing a research environment rich in high quality data.
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> Since engineering concepts and economic projections will be dependent on process analytical data, a clear QA/QC trail should be established linking high-throughput analysis methods to rigorous scientific and statistical standards. This can most easily be accomplished by establishing industry-wide consensus methods and publishing them through existing standards organization such as American Society for Testing and Materials (ASTM).

The response to this new special session topic was overwhelmingly positive, clearly indicating a need for increased research focus on analytical methods and forums for presenting innovative analytical methods used in fuels and chemical production.